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Characterization of Phytophthora cinnamomi from ornamental crops in South Carolina

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Characterization of *Phytophthora cinnamomi* from ornamental crops in South Carolina

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Introduction

Phytophthora cinnamomi Rands is a devastating pathogenic species of *Phytophthora.* It infects between 900 and 3,000+ hosts—many of which are ornamental plants. In the southeastern USA, it is the most common species causing root rot on woody nursery crops. As such, it is important to have an estimation of the diversity of *P. cinnamomi*. A better understanding of its genetic and morphological diversity will provide insight into the potential for this pathogen to adapt to current management strategies and could reveal cryptic groups within the species.

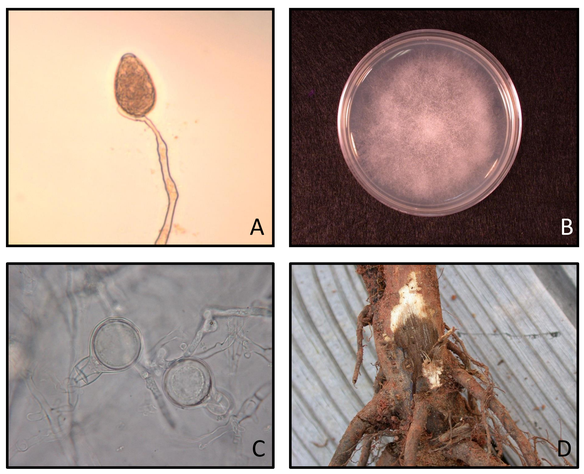

Figure 1. Morphology and symptoms of *P. cinnamomi:* **A**, a typical, non-papillate, ovoid sporangium in water; **B**, mycelium growth on PARPH-V8 after 72 h; **C**, a smooth-walled oospore in an oogonium with a 2-celled antheridium at the base of the oogonium; **D**, a canker on *Castanea dentata* (American chestnut) symptomatic of *P. cinnamomi* root rot.

Objective

Measure variation in key morphological and genetic characters among 142 isolates of *Phytophthora cinnamomi* recovered from ornamental crops between 1996-2011

Methods

Colony diameter and mycelium morphology

- Cultures were incubated on PARPH-V8 agar at 25C for 72 h in the dark
- Colony diameters were measured along two perpendicular transects and the values were averaged
- Morphology was sorted into four categories: aerial, appressed, sparse, and dwarf (figure 2)

Mefenoxam sensitivity

- Cultures were grown either on non-amended cV8A (controls) or on cV8A amended with 100 ppm mefenoxam
- Mycelium growth was rated visually from 0-5
- A mean rating of <4 indicated an isolate was sensitive to mefenoxam

Acknowledgments

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Methods

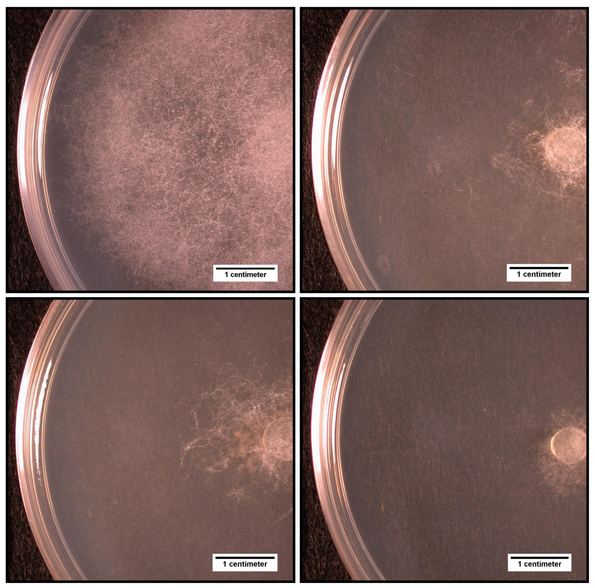


Figure 2. Mycelium types, clockwise from top left: **Aerial** – dense mats of hyphae growing above the agar surface, filling the space between the agar surface and the petri dish lid; **Appressed** – lack of aerial growth with hyphae on or below the agar surface; **Sparse** – similar to aerial but with a lower density of hyphae; **Dwarf** – extremely slow growth with dense mats of aerial hyphae.

Mating type

- Test isolates were paired with standard isolates of known mating type (A1 and A2) on super clarified V8A
- Isolates that produced oospores with the A1 standard were designated A2 and those that produced oospores with the A2 standard were designated A1

ITS sequence

- DNA was extracted and the ITS region was amplified
- MEGA 5.1 was used to align the sequences and build a neighbor-joining tree

Host-pathogen association

- Associations from this study group were compared to those in the literature
- Previously unreported host-pathogen associations were identified

Results

Colony diameter and mycelium morphology

Morphology type	Isolates (out of 142)		Colony diameter (mm)	
	no.	%	mean	median
Aerial	120	85	64.1	68.9
Sparse	17	12	34.9	32.4
Appressed	3	2	47.4	51.3
Dwarf	2	1	19.6	19.6

Mefenoxam sensitivity

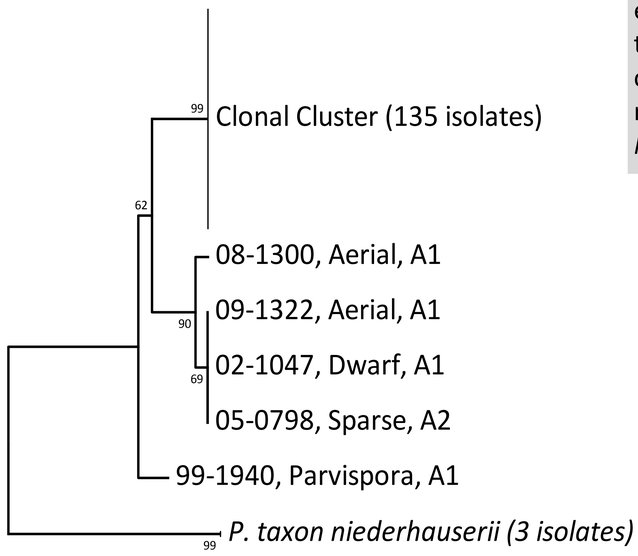
Sensitive	Resistant
142	0

Mating type

A1	A2
129	13

Results

ITS sequence and phylogeny for 140 isolates



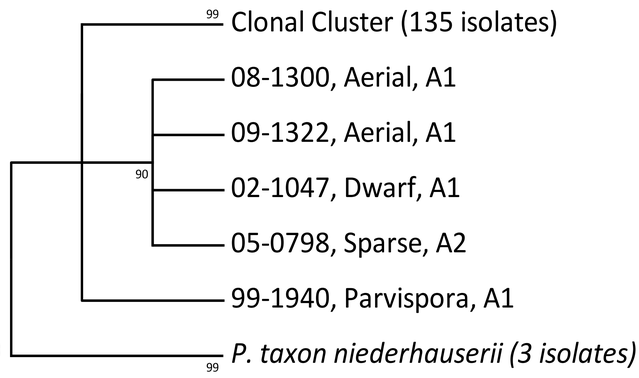


Figure 3. ITS phylogenies: The evolutionary history was inferred using the Neighbor-Joining method, and bootstrap values are shown on the branches. The analysis involved 143 nucleotide sequences: all positions containing gaps and missing data were eliminated, so there were a total of 589 positions in the final dataset. Evolutionary analyses were conducted in MEGA5: **Left**, uncondensed tree; **Right**, nodes with bootstrap values <75 are condensed. *Note:* Two isolates failed to sequence.

33 previously unreported host-pathogen associations

Host plant					Number of samples by county in SC or another state as indicated
Family	Genus	Species	Cultivar	Common Name	
Adoxaceae	*Viburnum*	*obovatum*		Small-leaf arrowwood	Hampton-1
	Viburnum	*tinus*		Laurustinus	Dorchester-1
Aquifoliaceae	*Ilex*	*cornuta*	Steeds	Chinese holly, horned holly	Laurens-1
Asteraceae	*Santolina*	*chamaecyparissus*		Cotton lavender, gray santolina	Albemarie, VA-1
Betulaceae	*Betula*	*nigra*		River birch	Dekalb, GA-1
Crassulaceae	*Sedum*	*reflexum*		Stonecrop	York-1
Cupressaceae	*Chamaecyparis*	*thyoides*		Atlantic white cypress	Oconee-1
	Cupressocyparis	*× leylandii*		Leyland cypress	Anderson-3, Georgetown-1, Greenville-1, Horry-1, Laurens-1, Pickens-1, Richland-2, Spartanburg-1
	Thuja	*occidentalis*	Emerald Green	Eastern arborvitae, northern white cedar	Pickens-1
Ericaceae	*Rhododendron*	hybrid	Encore	Azalea	Charleston-1
	Rhododendron	hybrid	Lord Roberts	Azalea	Abbeville-1
	Rhododendron	hybrid	Pink Ruffle	Belgian indica hybrid azalea	Greenville-1
	Rhododendron	hybrid	Yaku Princess	Azalea	Greenville-1
	Rhododendron	*× chinsei*	Chinsey, Chinsei	Chinsei hybrid azalea	Lexington-1
Hamamelidaceae	*Fothergilla*	sp.		Witchalder	Pickens-1
Hydrangaceae	*Hydrangea*	*quercifolia*	Alice	Oakleaf hydrangea	Pickens-2
Iteaceae	*Itea*	*virginica*	Little Henry	Virginia sweetspire	Pickens-1
Oleaceae	*Osmanthus*	*heterophyllus*	Verigated	Holly osmanthus	Charleston-1
Paeoniaceae	*Paeonia*	sp.		Peony	Lexington-1
Pinaceae	*Picea*	*glauca*		White spruce	Greenville-1
Pittosporaceae	*Pittosporum*	*tobira*	Dwarf	Japanese cheesewood	Georgetown-1
Plantiginaceae	*Digitalis*	*purpurea*	Excelsior	Common foxglove, purple foxglove	Pendleton-1
	Veronica	*officinalis*	Minuet	Gypsyweed	York-1
Rosaceae	*Eriobotrya*	*japonica*		Loquat, japanese medlar	Beaufort-1
	Photinia	sp.		Photinia	Richland-1
	Rhaphiolepis	*umbellata*	Blueberry Muffin	Indian hawthorn	Spartanburg-1
	Rosa	*banksiae*	Lady Banks Rose	Lady banks' rose	Lexington-1
Rubiaceae	*Gardenia*	sp.		Gardenia	Beaufort-1
Sapindaceae	*Acer*	*saccharum*	Green Mountain	Sugar maple	Florence-1
Schisandraceae	*Illicium*	*floridanum*		Star-anise, purple-anise	Oconee-1
Styracaceae	*Halesia*	*tetraptera (syn. carolina)*		Carolina silverbell	Charleston-1
Theaceae	*Cleyera*	*japonica*		Sakaki	Pickens-1
	Ternstroemia	*gymnanthera*		Japanese ternstroemia	Georgetown-1

Conclusions

This population of 142 *P. cinnamomi* isolates from ornamental crops is fairly homogenous in morphology and genetics. Only two clades were distinct from the main clade that contained 135 of the isolates. One clade consisted of a single isolate of *P. cinnamomi* var. *parvispora*, while the other clade contained a morphologically diverse set of four isolates. More genetic information is needed to measure the diversity of this population and to determine more meaningful relationships between morphology and genotype.